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The effects of prenatal stress on temperament and problem behavior of 27-month-old toddlers

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Abstract *Aim* To examine, in a prospective study, the influence of prenatal stress on infant temperament and problem behavior. *Method* Self-report data on stress and anxiety, and levels of cortisol in saliva were collected from nulliparous women during pregnancy. Temperament of the child was measured at 27 months by parent report on the Infant Characteristics Questionnaire. Behavior of the child was assessed by direct observation during the administration of the Bayley Scales of Development 2–30, and by parent report on the Child Behavior Checklist 2–3. *Results* Complete data were available for 103 healthy toddlers. Logistic regression analyses were performed and results were adjusted for possible prenatal, perinatal and postnatal confounders. Perceived stress during pregnancy was a predictor of lower levels of restless/disruptive temperament (OR = 0.77), more total behavioral problems (OR = 1.17), and more externalizing behavioral problems (OR = 1.12) in 2-year-olds. Fear of bearing a handicapped child was a predictor of higher levels of restless/disruptive temperament (OR = 1.39) and more attention regulation problems in toddlers (OR = 1.46). *Conclusions* Increased levels of maternal prenatal stress appear to be associated with temperamental and behavioral problems in toddlers.

Key words prenatal stress – toddler – temperament – behavior

Introduction

There is accumulating evidence that maternal stress during pregnancy, probably in interaction with genetic factors, may have long-lasting adverse consequences on the brain and the behavioral development of the offspring [3, 64]. The most direct evidence comes from animal studies of prenatal exposure to a wide variety of physical stressors, such as repeated electrical tail shock [53], immobilization [62], noise [11, 49] and saline injections [13, 39], as well as different forms of social stress [3, 14, 45, 52].

The negative effects include increased levels of anxiety, decreased exploration of novel or intimidating environments, and decreased adaptation to other postnatal stressful conditions [43, 51, 53, 66]. Furthermore, prenatally stressed animals have a reduced propensity for social interactions, impaired sexual functioning, and poor performance in various learning paradigms [40, 44, 54]. Finally, adverse effects of prenatal stress on physical development have also been documented, such as lower birth weight, delayed motor development, and higher death rate in the perinatal period [15, 30, 46, 65].

Prenatally stressed offspring appear to have an impaired regulation of the hypothalamic-pituitary-adrenal (HPA) axis [5]. For example, they have higher baseline levels of glucocorticoids; a faster, stronger, and more prolonged response to stress and diminished feedback sensitivity; changes in neurotransmitter systems;

and a reduced cerebral asymmetry and turnover of dopamine [4, 19, 21, 63].

Research on maternal stress in humans has mostly relied on retrospective assessment of psychological stress or anxiety during pregnancy. The results mainly indicate that prenatal stress is related to poor birth outcome (lower birth weight, premature delivery, and a small head circumference) [16, 33]. The few existing prospective studies show an association of prenatal stress with difficult temperament at 10 weeks and 7 months after birth, and with problem behavior, anxiety and lower attention regulation at 8–9 years of age [55, 56], and more behavioral and emotional problems in 4-year-old children [35]. Prenatal exposure to major disasters, such as earthquakes, floods, or war, has been found to be associated with increased psychopathology in the offspring [17, 28, 48].

This article reports on the results of an ongoing prospective longitudinal study of the effects of prenatal stress in humans on the cognitive and behavioral development of children [20, 21, 23]. Stress was assessed by self-report and hormone measurements in early, mid and late pregnancy, using a multidimensional concept of stress according to the model of Lazarus and Folkman [29]. This model describes a differentiation between three types of factors: stress-provoking factors, such as daily hassles and life events, stress-mediating factors, such as coping and social support, and stress resulting factors, such as anxiety and perceived stress.

Our previous report has shown that high levels of pregnancy-specific anxiety predicted less test-affectivity and goal-directedness when the offspring were 8 months, whereas perceived stress predicted greater 'unadaptability' on the Infant Characteristics Questionnaire, a measure of temperament, at 3 months [23]. Maternal levels of ACTH during mid-pregnancy were associated with greater unadaptability at 8 months. Thus, the effects of prenatal stress on temperament appeared to be more clear-cut at 8 months than at 3 months of age. Prenatal daily hassles were not related to temperament at 3 and 8 months. In the present paper, we present our findings on the relations between prenatal stress and temperament and problem behavior in the same cohort at 2 years of age.

In line with our previous findings [23] and those of other researchers in the field [35, 55, 56], we expect toddlers whose mothers reported more stress during pregnancy to have a more difficult temperament, poorer attention regulation, and more problematic behavior than toddlers whose mothers reported lower amounts of stress. Furthermore, on the basis of the model of Lazarus and Folkman, we expect stress-resulting factors to be the best predictors of temperamental and behavioral problems in 27-month-old toddlers.

Early morning cortisol and the mean level of cortisol did not predict temperamental problems at 3 or 8 months [20]. Therefore we do not expect cortisol to play a role in temperament and problem behavior in 27-month-old toddlers.

Methods

Subjects

This study is part of an ongoing prospective longitudinal project that investigates the possible influence of prenatal psychosocial factors on fetal behavior and postnatal development. The 230 subjects were recruited from a population of pregnant women who visited the Outpatient Clinic of the Department of Obstetrics of the University Medical Center Utrecht, The Netherlands, between January 1996 and July 1998. All women were expecting their first singleton child and participated on a voluntary basis. The local ethics committee approved the study and the subjects gave written informed consent. Only healthy infants born after 37 weeks of gestation were included in the follow-up study.

Of the 230 women who completed the prenatal questionnaires on the first occasion, 217 completed the questionnaires on the second occasion, and 172 on the third occasion. The main reasons for dropout were personal reasons, such as moving away, lack of interest, lack of time. Of the 172 mother-child pairs that participated at the third occasion, 28 were excluded because of medical reasons, such as preterm delivery (N = 18), stillbirth, and serious pregnancy complications, and three because they delivered twins. One child died after birth. Of the remaining 140, 119 mother-child pairs agreed to participate when the child was 27 months old (53 % of the babies were female). Again, the main reasons not to participate for the remaining 21 mothers were personal. There was no selective attrition between the 119 mother-child pairs who participated and the 21 who did not. They were similar in all the measured prenatal stress levels, gender, maternal age, SES, mental and motor development and temperament measured at the age of 8 months.

Prenatal maternal stress levels

Psychosocial measures

Four different psychosocial stress scores were measured during and after pregnancy. The first was a stress-provoking factor: the frequency of daily hassles, measured with the Everyday Problem List [58]. This questionnaire is based on different questionnaires: the Daily Life Experience Questionnaire [50], the Daily Hassles Scale [25] and the Everyday Problem Scale [10].

The second stress score was pregnancy-related anxi-

ety (a stress resulting factor), assessed by means of the Pregnancy Related Anxiety Questionnaire-Revised, PRAQ-R [55]. For this study, two Dutch scales were used: fear of bearing a physically or mentally handicapped child (4 items) and fear of giving birth (3 items) [22].

The third stress score was perceived stress (stress resulting factor), measured with the Dutch version of the Perceived Stress Scale of Cohen & Williamson [12].

The fourth stress score was psychological well-being, determined using the Dutch translation [26] of the General Health Questionnaire-30 (GHQ-30) [18]. This questionnaire contains 30 questions scored on a 4-point scale, ranging from 'never' to 'always'. The items assess the degree to which the mothers perceived their lives as unpredictable, uncontrollable, and burdensome.

The correlations of the psychological stress measures between the three pregnancy periods (15–17 weeks, 27–28 weeks, and 37–38 weeks of gestation) were strong and significant (range 0.38–0.75, p's < 0.01) for all questionnaires. Thus, to reduce the chance of a type I error (rejection of the null-hypothesis, while it is actually true), the number of variables was reduced by using the mean scores over pregnancy. Correlations between prenatal psychosocial variables were in general low (range 0.04–0.41), except for the correlation between perceived stress and psychological well being (r = 0.66).

Endocrinological measure

Maternal salivary cortisol levels were determined as a measure of HPA-axis function. Samples were collected in each of the three periods of pregnancy (15–17 weeks, 27–28 weeks, and 37–38 weeks of gestation) on a preselected day every 2 hours between 8 AM and 8 PM to obtain day curves.

As a consequence of the size of the study population and the high amount of variables, and with the object of avoiding Type I errors, we did not take all the gathered information into account. Therefore, we chose to take only the two values that were also used in an earlier study on the same cohort, when the children were 3 and 8 months old [23]. These were the mean values of the day curves and the 8 AM samples, calculated for each of the three pregnancy periods.

Toddler measures

Temperament: Infant Characteristics Questionnaire (ICQ)

The shortened version of the Dutch translation [27] of the Infant Characteristics Questionnaire [8] contains 24 items and assesses difficult behavior, adaptability, and attention regulation. Difficult behavior refers to a negative mood, withdrawal, high intensity, and low regularity of physical rhythms. Adaptability reflects adaptation to novel situations and attention regulation reflects task persistence and attention span [8]. On 7-point Likert scales mothers fill out how difficult they experience the toddler's temperament to be. Score 0 corresponds with easy temperament (for example: child does not often get upset, does not often do unexpected things, is easy to calm/soothe) and score 7 corresponds with very difficult temperament (for example: child gets upset very often, often does unexpected things, is difficult to calm/soothe).

The ICQ was chosen in order to be able to compare the results with those found at the ages of 3 and 8 months [23]. Although normative and factor structure data are available for the Dutch population at 3 and 8 months, they are not available for the age of 27 months. Therefore, an exploratory factor analysis (varimax rotation) was performed, resulting in four factors. Two factors, representing restless/disruptive behavior, correlated high, as did the other two factors, representing irritable behavior. Therefore, another factor analysis was performed, in which the number of factors was restricted to two. Only items loading more than 0.40 on a factor were included. This resulted in the factors 'Restless/disruptive behavior', which was assessed by 12 items and explained 19 % of the variance, and 'Irritability', which refers to negative emotionality, was assessed by 11 items, and explained 16.3 % of the variance. Details of the factor analyses can be obtained from the first author on request. Cronbach's alpha scores were 0.83 and 0.80, respectively. Although the Pearson correlation between the two factors was 0.49 (p < 0.01), the two factors were used separately, as they appeared to be two different constructs based on the items loading on each factor. The items of the two factors are presented in Table 1.

Attention regulation: Bayley Scales of Infant Development 2–3 (BSID 2–30)

Two trained psychologists, blinded with respect to the prenatal maternal stress levels, determined the third component of the Bayley Scales of Infant Development 2–30 [9] when the child was 27 months old. This instrument was used because it provides an objective and reliable way of measuring behavior.

The behavior of the child was directly observed during testing in a standard laboratorium situation, and was coded on the Infant Behavior Record. An aggregated sum score of the subscales Goal-Directedness and Test-Affectivity resulted in the variable attention regulation [57]. The items that form this scale are listed in Table 2. To check reliability, the two observers independently recoded 20 % of the observations. The resulting mean inter-rater reliability with the original codes was > 0.89 (Cohen's kappa).

European Child & Adolescent Psychiatry (2005) Vol. 14, No. 1

Table 1 Loadings of the items of the ICQ on the two factors

Item	Restless/disruptive behavior	Irritability
On the average, how much attention does your child require, other than for caregiving (feeding, diaper changes, etc.)?	0.67	
How active is your child in general?	0.67	
**Is it easy or difficult for your child to sit still, for example, during dinner?	0.59	
When left alone, your child plays well by himself/herself?	0.65	
**Is your child easily distracted? For example, by noise?	0.78	
**Is your child often impatient? Does it have trouble waiting?	0.40	
Please rate the overall degree of difficulty your child would present for the average mother	0.50	0.41
Does your child persist in playing with objects when he/she is told to leave them alone?	0.58	
**Does your child often not seem to register the things you say to him/her?	0.48	
**Does your child often change from activity? For example, while playing?	0.69	
**When your child wants something, is he/she persistent in achieving his/her goal? (by this we mean when the child is making efforts to achieve something, and not when he/she is whining for something)	0.41	
**Does your child often do unexpected things?	0.41	
How easily does your child get upset?		0.64
When your child gets upset, how vigorously or loudly does he/she cry and fuss?		0.48
How easy is it for you to calm or soothe your child when he/she is upset?		0.53
When removed from something he/she is interested in but should not be getting into, your child gets upset.		0.55
How does your child react to being confined (As in a carseat, infant seat, playpen etc.)		0.44
How changeable is your child's mood?		0.64
How consistent is your child in sticking to his/her sleeping and/or eating routine?		0.52
How well does your child adapt to new experiences?		0.48
**Do you consider your child easy or difficult?		0.66
How much does your child cry and fuss in general?		0.72

** items that were added in the Dutch version of the ICQ

Table 2 Items that create the scale 'attention regulation'

Goal-directedness	Test-affectivity
Object orientation	Endurance
Goal-directedness	Emotional tone
Attention span	Social examiner
Reactivity	Cooperativeness
	Fearfulness

Problem behavior: the Child Behavior Checklist 2–3 (CBCL 2–3)

The mothers also filled out the Dutch version of the CBCL 2–3 [1, 2]. This list contains 99 items that describe a large number of different behavioral problems. The instrument was chosen because it is a well-known and widely used instrument to measure problem behavior. Scores were 0 (option was never true for the child), 1 (option was occasionally true), and 2 (option was often true). The answers to the 99 items resulted in scores for the following seven behavioral categories: oppositional, aggressive, overactive, withdrawn, anxious, sleep problems, and somatic problems. Three higher order problem categories were used as dependent variables: the total internalizing problem factor, which is the sum of the behavioral categories withdrawn and anxious; the total externalizing problem factor, which is the sum of the first three categories (oppositional, aggressive and overactive); and the total problem factor, which is the sum of all 7 behavioral problem categories.

Potential covariates

Prenatal, perinatal, and postnatal data were collected to control for factors that might confound the effects of prenatal stress: maternal age in years, smoking behavior, use of alcohol, socioeconomic status (SES), prenatal risk factors, perinatal factors, postnatal factors, and gender.

At the time of birth, the mean maternal age (± SD) was 32.0 ± 4.8 years. Smoking behavior, measured in the three periods of pregnancy, was divided into two groups: 1) non-smokers, and 2) smokers, i. e. at least 1 cigarette a day (n = 15 in the first and the second trimester, and 11 in the third trimester). Alcohol intake was also divided into two groups: non-drinkers and drinkers, i. e. more than 1 drink a week (n = 18 in the first trimester, 24 in the second trimester, and 26 in the third trimester). Only 11 subjects consumed more than 2 alcoholic drinks per week at one or more trimesters. Educational and professional levels of the pregnant mother and her partner were scored and used to check SES. Three levels of education were determined: low (primary school, high school education), middle (secondary school education), and high (college or academic education). Prenatal biomedical risk factors were scored as "absent [0]" or "present [1]" and included pregnancy complications (n = 22; 18.5 % (i. e. pre-existent health problems (n = 11; 9.2 %), high blood pressure (n = 8; 6.8 %), and gestational diabetes mellitus (n = 3; 2.5 %)), use of medication during pregnancy (n = 17; 14.3 %), fertility problems (n = 37; 31.1 %), in vitro fertilization (n = 8; 6.7 %), and gynecological risks (n = 5; 4.2 %). Cumulative risk scores were calculated resulting in a categorical variable. Perinatal factors taken into account were birth weight, gestational age, and complications during delivery. Mean birth weight was 3378 ± 492 grams and mean gestational age was 40 ± 1.3 weeks. Complications during delivery were intrapartum complications (n = 21; 17.6 %), use of medication during delivery (n = 65; 54.6 %), elective cesarean section (n = 14; 11.8 %), and assisted delivery due to fetal distress (n = 16; 13.4 %). These perinatal complications (0 = absent; 1 = present) were calculated as a cumulative score and transformed into a categorical variable.

Finally, postnatal factors were obtained when the child was 27 months old by using the same questionnaires described above: General Health Questionnaire (GHQ), perceived stress (VES) and daily hassles (APL).

Statistical analyses

The majority of children in this study did not show clinically meaningful levels of temperamental, behavioral or attention problems. Consequently, because we were interested in whether the findings were of potential clinical relevance and comparable with results described by O'Connor et al. [35], we analyzed the data with logistic regression. In this way we could determine whether the toddlers with the most temperamental and behavioral problems were those whose mothers had experienced the greatest prenatal stress. Logistic regression also provides the opportunity to correct for several possible covariates [35].

Subjects who scored ≥ 1 SD above the mean for the 'restless/disruptive behavior' and 'irritability' scales of the ICQ and for the internalizing, externalizing, and total problem scales of the CBCL 2–3 were given a score of 1 and the remaining subjects a score of 0. The variable attention regulation (BSID 2–30) was recoded as follows: subjects who scored ≤ 1 SD below the mean were given a score of 1 and the remaining subjects were given a score of 0. Attention regulation was now called 'attention regulation problems'.

The logistic regressions were performed with backward Wald analyses and odds ratios (OR) are reported as the index of association. A hierarchical approach was used: all possible confounders were entered in step 1 and the prenatal stress factors in step 2. The ICQ factors, the CBCL 2–3 and the Bayley Scales were the dependent variables.

Results

Descriptive analyses

Descriptive SES information on the sample is provided in Table 3. Most of the women were middle class, although both lower and higher social classes were represented. At study inclusion, most women lived with their partner, either in wedlock or unmarried (97.5 %) and had a paid job (84 %). To check for possible selective attrition, the prenatal, the available postnatal data of the mothers and the available data of the children of the group of mothers and children who participated in the study were compared with the data of the non-participating group. There were no significant differences between the participating and the non-participating group.

In Table 4 the means and standard deviations of the predictors and dependent variables are presented, together with norm values for the predictors. As can be

Table 3 Socioeconomic status of the subjects

Level	Education		Profession	
	Mother	Partner	Mother	Partner
Low	10.3 %	22.4 %	6 %	16.4 %
Middle	69.2 %	58.6 %	53.5 %	28.4 %
High	20.5 %	19 %	40.5 %	55.2 %

European Child & Adolescent Psychiatry (2005) Vol. 14, No. 1

Table 4 Means and standard deviations of the predictors and the dependent variables

	N	Mean	SD	Norm values
Predictors				
Daily hassles (APL)	118	8.1	4.7	< 9 is normal 10–16 is high > 17 is very high[1]
Fear of giving birth	104	5.7	2.3	No norm values available
Fear of bearing a handicapped child	104	8.6	2.6	No norm values available
Psychological well being (GHQ)	118	4.5	3.8	Mean values of 2.72 and 4.51 were found in normal populations[2]
Perceived stress (VES)	118	27.1	4.9	Normal population; mean = 20.2, sd = 7.8[3]
Mean cortisol	103	13.1	2.9	Comparable values were found in other pregnant populations[4]
Early morning cortisol	115	22.0	5.1	Comparable values were found in other pregnant populations[4]
Dependent variables				
'Restless/disruptive'	111	42.2	9.0	No norm values available
'Irritability'	112	36.1	8.2	No norm values available
Attention regulation	112	57.3	7.8	Normal population; mean = 52.4[5]
Total problems of the CBCL 2–3	110	48.9	8.4	Clinical cut off = 70[6]
Internalizing problems	110	46.4	8.6	Clinical cut off = 70[6]
Externalizing problems	110	48.8	7.3	Clinical cut off = 70[6]

[1] Norm values APL (Vingerhoets et al., 1989); [2] Koeter & Ormel, 1991; [3] Cohen & Williamson, 1987; [4] Sikkema, Robles de Medina, Schaad, Mulder, Bruinse, Buitelaar, Visser, & Franx, 2001; [5] Van der Meulen & Smrkovsky, 1983; [6] Achenbach, Edelbrock, Howell, 1987

seen in the table, the study population shows non-clinical scores on all the variables.

With respect to the correlations between the dependent variables, the factors of the ICQ (restless/disruptive behavior and irritability) were poorly to moderately related to the CBCL scores. The significant relations were for restless/disruptive behavior with externalizing and total problem behavior: r = 0.45 and r = 0.29, respectively; and for irritability with externalizing, internalizing, and total problem behavior: r = 0.50, r = 0.37, and r = 0.50, respectively (all p-values < 0.01). Finally, attention regulation was modestly correlated with problem behavior (range 0.27 to 0.31, p's < 0.05) and not correlated to restless/disruptive behavior and irritability (ICQ).

Prenatal maternal stress, child temperament, and problem behavior at 27 months

The results are summarized in Table 5. Logistic regression analysis with temperamental 'restless/disruptive behavior' (ICQ) as the dependent variable, the prenatal psychosocial stress measures as predictors, and adjusted for confounders, showed two effects of prenatal stress. Prenatal fear of bearing a handicapped child was significantly related to restless/disruptive temperament. Children of mothers who reported more fear of bearing a handicapped child, had 1.39 more chance to be in the restless/disruptive temperament group, compared to children whose mothers reported less fear of bearing a handicapped child. In contrast, lower levels of perceived stress during pregnancy were associated with restless/disruptive temperament (OR = 0.77, 95 % CI 0.61–0.99). Several confounders were also associated with restless/disruptive temperament. Smoking during the first trimester of pregnancy and postnatal perceived stress were associated with higher levels of 'restless/disruptive behavior' when the children were 27 months old (OR = 36.61; 95 % CI 3.86–346.88, p = 0.01 and OR = 1.45; 95 % CI 1.09–1.91, p = 0.01). Furthermore, older mothers and mothers who had higher scores on the general health questionnaire when their child was 27 months old, reported their child as less restless/disruptive (OR = 0.83; 95 % CI 0.70–0.99, p = 0.03 and OR = 0.78; 95 % CI 0.61–0.99, p = 0.05).

Logistic regression analysis with temperamental irritability (ICQ) as the dependent variable, the prenatal psychosocial stress measures as predictors, and adjusted for confounders, showed that maternal age was the only predictor of extreme scores for 'irritability' in children at 27 months (OR 0.93; 95 % CI 0.87–1.00, p = 0.05). Older mothers reported their child as less irritable.

Logistic regression analysis with attention regulation problems (BSID-2–30) as the dependent variable, the prenatal psychosocial stress measures as predictors, and

Table 5 Logistic regression analyses of pre-, peri-, and postnatal factors on temperament and problem behavior in 2-year-olds

	β	OR (95 %CI)	WALD	P
'Restless/disruptive behavior' (ICQ)				
Perceived stress during pregnancy	–0.26	0.77 (0.61–0.99)	4.29	0.04
Fear of bearing a handicapped child	0.33	1.39 (1.01–1.92)	4.13	0.04
Maternal age	–0.19	0.83 (0.70–0.99)	4.58	0.03
Postnatal psychological non well-being	–0.25	0.78 (0.61–0.99)	3.91	0.01
Postnatal perceived stress	0.37	1.45 (1.09–1.91)	6.63	0.05
Smoking during the first period of pregnancy	3.60	36.61 (3.86–346.88)	9.85	0.01
Irritability (ICQ)				
Maternal age	–0.07	0.93 (0.87–1.00)	3.84	0.05
Attention regulation problems (BSID 2–30)				
Fear of bearing a handicapped child	0.38	1.46 (1.07–1.99)	5.66	0.02
Maternal age	–0.17	0.85 (0.72–0.99)	4.46	0.04
Postnatal psychological non well-being	0.28	1.32 (1.09–1.61)	7.68	0.01
Postnatal perceived stress	–0.27	0.76 (0.61–0.96)	5.56	0.02
Total problem behavior (CBCL)				
Perceived stress during pregnancy	0.16	1.17 (1.05–1.31)	7.79	0.01
Externalizing problem behavior (CBCL)				
Perceived stress during pregnancy	0.12	1.12 (1.00–1.26)	3.88	0.05
Smoking during the first period of pregnancy	1.54	4.65 (1.22–17.73)	5.07	0.02
Internalizing problem behavior (CBCL)				
Postnatal psychological non well-being	0.21	1.24 (1.09–1.40)	10.63	0.01

adjusted for confounders showed that higher prenatal levels of fear of bearing a handicapped child were related to higher levels of attention regulation problems in 2-year-olds (OR = 1.46; 95 % CI 1.07–1.99, p = 0.02). Furthermore, older mothers had children with lower levels of attention regulation problems (OR = 0.85; 95 % Cl 0.72–0.99, p = 0.04). Postnatal levels of stress also played a role in attention regulation of the 27-month-old children. Higher postnatal maternal scores on the General Health Questionnaire were related to higher attention regulation problems of the children (OR = 1.32; 95 % CI 1.09–1.61, p = 0.01). In contrast, higher levels of postnatal perceived stress were related to lower levels of attention regulation problems (OR = 0.76; 95 % CI 0.61–0.96, p = 0.02).

Logistic regression analyses with the three behavioral factors (total problem behavior, externalizing problems, and internalizing problems of the CBCL 2–3) as dependent variables, the prenatal psychosocial stress measures as predictors, and adjusted for confounders showed that perceived stress during pregnancy was related to more total problem behavior (OR = 1.17; 95 % CI 1.05–1.31, p = 0.01) and more externalizing problems (OR = 1.12; 95 % CI 1.01–1.26, p = 0.05). Externalizing problems were also related to smoking during the first period of pregnancy (OR = 4.65; 95 % CI 1.22–17.73, p = 0.02), and postnatal levels of stress (general health questionnaire) played a role in internalizing problem behavior, with higher levels of postnatal stress being associated with more internalizing problems (OR = 1.24; 95 % CI 1.09–1.40).

Prenatal maternal cortisol levels, child temperament, and problem behavior at 27 months

In logistic regression analyses both with and without psychosocial measurements (the questionnaires), maternal cortisol level proved to be unrelated to temperamental and behavioral problems.

Discussion and conclusions

This study showed various aspects of prenatal maternal stress to be independently associated with temperamental and behavioral outcome in 27-month-old toddlers. Though the relations between prenatal stress and temperament and problem behavior were not very strong, it should be kept in mind that the sample of this study had relatively low amounts of stress during pregnancy.

As we had hypothesized, the most clear-cut results on temperament and behavior were found when the prenatal stress factors were stress-resulting factors (i. e. perceived stress and anxiety) instead of stress-provoking factors (i. e. daily hassles). Some researchers have suggested that daily hassles can be more harmful than other stress factors (e. g. [38]). Reasons for not finding an effect of daily hassles in our sample could be that the mothers experienced relatively normal levels of daily hassles [58], that our sample had relatively normal psychosocial risk factors, and that most of the mothers had a middle class background. Daily hassles may play a

more important role in mothers exposed to more daily hassles, in those with lower socioeconomic status, or in those at risk of premature birth. However, our results appear to indicate that in a non-clinical population, when the influence of the subjectively perceived and resulting levels of stress are taken into account, the variable daily hassles does not contribute to the prediction of later temperament and problem behavior.

Prenatal maternal anxiety, in the form of fear of bearing a handicapped child, was related to temperament and attention regulation. Children whose mothers reported more fear of bearing a handicapped child had more chance of being in the restless/disruptive behavior group (ICQ) and in the more attention regulation problems group (BSID 2–30). Interestingly, while temperament scores were based on maternal report, attention regulation scores were based on observations made by an independent experimenter. This diminishes the chance that the results were obtained solely due to report bias. The fact that similar relations had already been found when the children were 3 and 8 months of age [20], suggests long-lasting effects. Finally, the results are in line with those found in the large study (n = 7448) by O'Connor and colleagues on the influence of prenatal maternal anxiety on behavioral and emotional problems in 4-year-old children [35]. Therefore, the present study adds to the accumulating evidence that specific pregnancy anxiety, in the form of fear of bearing a handicapped child, is an important component of a pregnant woman's emotional state and a predictor of pregnancy outcome and later temperamental and behavioral development [23, 41, 59, 60].

In contrast to pregnancy anxiety, higher levels of perceived stress during pregnancy were associated with less restless/disruptive behavior (ICQ) in the toddlers. Earlier in this longitudinal project, when the children were 3 months old, a significant positive relationship between prenatal perceived stress and difficult behavior was found [23]. At 8 months this effect was reduced to a trend [23] and in the current assessment at 27 months the relationship with perceived stress is in the opposite direction. A possible explanation for this intriguing result is that at the 3 and 8 months analyses a total score of the ICQ was used, and no differentiation was made between restless/disruptive behavior and irritability. This might explain the differences in results. And indeed, post hoc analyses with the total score of the ICQ at 27 months of age as the dependent variable did not show an effect of prenatal perceived stress at all, which is in line with our previous findings.

In addition to being related to less restless/disruptive behavior, higher levels of prenatal perceived stress were found to be associated with toddler problem behavior. More perceived stress during pregnancy was related to more total problems and more externalizing problems (CBCL 2–3) in 27-month-old children. Easy frustration, more crying, and more angry moods characterized the problem behavior. Once again, these results are consistent with those of O'Connor et al. [35], who found that prenatal maternal anxiety (at 32 weeks of pregnancy) was related to more emotional problems and more conduct problems in children at age 4.

The fact that perceived stress, both prenatal and postnatal, shows such contrasting relations with the temperament factors, observed attention regulation problems and problem behavior is difficult to explain. Naturally, the nature of the dependent variables is different: while one is based on behavioral observations of an experimenter (BSID 2–30), another is the mother's reported perception of behavior (ICQ), and the others are based on maternal reports of concrete behavioral problems (CBCL). This most probably, at least partly, explains the different relationships found with perceived stress. However, a replication study is needed in order to see whether these results stand. The same is true for the contrasting findings on the postnatal psychological non-well-being scores (General Health Questionnaire).

Chronic maternal stress during pregnancy, associated with raised plasma levels of CRH, ACTH, and cortisol, may increase the likelihood of behavioral abnormalities in children [64]. In the present study prenatally increased cortisol levels in the mothers were not a predictor of temperamental characteristics or problem behavior in toddlers. Again, these findings are in line with our earlier findings at 3 and 8 months of age [23]. There are at least three possible explanations for this. First, it could be that cortisol levels do not adequately reflect a pregnant woman's physiological stress status. Other HPA axis hormones, such as CRH and ACTH, may be important and should be measured in future studies. Second, maternal cortisol levels may be associated with HPA axis function in the child, rather than with temperamental, behavioral, or attention regulation problems. This could be investigated by closely monitoring HPA axis function in children, by measuring cortisol levels and cortisol reactivity to different stressors. Third, the fact that stress-resulting factors showed relations with temperamental and behavioral problems, while prenatal maternal cortisol levels did not, could mean that these prenatal variables are unrelated and have different effects on the unborn child. This is supported by the fact that positive correlations between levels of cortisol and perceived stress have not always been found [34].

Apart from the prenatal stress effects, a number of confounding factors were associated with temperament and behavior. Toddlers of mothers who smoked during the first period of pregnancy had higher levels of 'restless/disruptive behavior' (ICQ) and more externalizing problem behavior (CBCL 2–3) compared to toddlers whose mothers had not smoked during pregnancy. Similar results were reported by Orlebeke, Knol, & Verhulst [36].

These effects need not only be physiological as, for example, Wakschlag et al. [61] have shown that women who continue to smoke during pregnancy also report more behavioral problems in the children. In our study, posthoc analyses showed a correlation between smoking and prenatal stress. However, both variables were independently related to postnatal outcome, each explaining their own part of variance. In our sample, smoking in the first trimester of pregnancy was related to restless/disruptive behavior and externalizing problem behavior.

Also, maternal age played a role in 'restless/disruptive behavior' (ICQ), 'irritability' (ICQ), and attention regulation (BSID 2–30). Children who had younger mothers had more problems than children with older mothers, which is consistent with previous studies showing maternal age to be related to temperament and problem behavior [e. g. 24, 37, 47].

The present study supports the increased awareness of the fetal environment as a determinant of health in later life. Parameters of the early life environment, notably low birth weight and thinness at birth predict later occurrence of ischemic heart disease deaths, hypertension and insulin-resistant type 2 diabetes [e. g. 6, 7]. These early life associations do not seem to be restricted to the field of somatic health, but appear to be relevant for behavioral development as well.

Study limitations

Our findings may be open to the criticism of informant bias, since a part of the prenatal stress measures and the reports on the temperamental characteristics and problem behavior of the children were obtained by maternal report. However, the fact that we obtained similar findings with independent laboratory observations of the toddlers' attention regulation, strengthens the value of the results based on the self-report data.

We did not use a genetic informed design and could therefore not investigate the influence of genetic factors on the temperament and behavior of toddlers and infants [23]. Maternal genetic vulnerability factors, which can be passed on to the child, are most probably partly responsible for both maternal stress and temperamental and behavioral difficulties in the toddler. Twin and sibling studies are needed to address the gene-environment issues.

As already mentioned, another limitation of this study is that our population was composed of pregnant women with relatively normal psychological risk factors, and a limited range of prenatal stress scores. This makes it difficult to generalize the findings to other populations of pregnant women. Lastly, because the mean scores of three prenatal stress assessment moments were used, it was not possible to study the influence of prenatal stress in the different periods of pregnancy on temperamental and behavioral outcome.

Clinical implications

The results of this study show associations between pregnancy-specific anxiety and perceived stress during normal risk pregnancies with temperamental and behavioral problems in term-born toddlers. Although follow-up studies are necessary to determine whether the effects are persistent, difficult temperament has been found to be associated with more problem behavior in later life [42]. Early externalizing problems can play a significant role in later development of internalizing problems [31], and both internalizing and externalizing problems can predict their *DSM-IV* counterparts 8 years later [32]. Our findings underline the importance of assessing stress-resulting factors in pregnant women. Information about stress factors can easily be obtained by means of maternal questionnaires. Pregnant women at increased risk could then be invited to participate, for example, in stress reduction programs.

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